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(54) **DNA fragment encoding a polypeptide having nitrile hydratase activity, a transformant containing the DNA fragment and a process for the production of amides using the transformant.**

(57) The present invention discloses the amino acid sequence and nucleotide sequence of the α- and β-subunits of two types of nitrile hydratase derived from Rhodococcus rhodochrous J-l. The DNA fragment encoding nitrile hydratase is inserted into an expression vector and the recombinant vector is used for transformation. The transformant contains multiple copies of the gene and can produce much higher levels of nitrile hydratase than conventionally used microorganisms.

The present invention relates to a DNA fragment derived from Rhodococcus rhodochrous J-l and encoding a polypeptide having nitrile hydratase activity which hydrates nitriles to amides. The invention also relates to a recombinant DNA containing the above DNA fragment, and a transformant transformed with the recombinant DNA. The present invention further relates to a method of producing nitrile hydratase using the transformant and of amides using nitrile hydratase.

Nitrile hydratase or nitrilase is known as an enzyme that hydrates nitriles to amides. Microorganisms that produce nitrile hydratase include those belonging to the genus Bacillus, the genus Bacteridium, the genus Micrococcus and the genus Brevibacterium (See, JP-B-62-21517/1989, USP No. 4,001,081), the genus Corynebacterium and the genus Nocardia (See, JP-B-56-17918/1981, USP No. 4,248,968), the genus Pseudomonas (See, JP-B-59-37951/1984, USP No. 4,637,982), the genus Rhodococcus, the genus Arthrobacter and the genus Microbacterium (See, JP-A-61-162193/1986, EP-A-0188316), and Rhodococcus rhodochrous (See, JP-A-2-470/1990, EP-A-0307926).

Nitrile hydratase has been used to hydrate nitriles to amides. In the invention, microorganisms are engineered to contain multiple copies of a recombinant DNA encoding nitrile hydratase according to a recombinant DNA technology. The recombinant produces a remarkably high level of nitrile hydratase compared with conventionally used microorganisms.

The present inventors previously disclosed a DNA fragment derived from Rhodococcus sp. N-774 (FERM BP-1936) which also encodes a polypeptide having nitrile hydratase activity (JP-A-2-119778/1988).

In contrast, the present inventors utilizes a DNA fragment derived from Rhodococcus rhodochrous J-l for the production of nitrile hydratase. We isolated the gene encoding nitrile hydratase, inserted the gene into a suitable plasmid vector and transformed an appropriate host with the recombinant plasmid, thus successfully obtained the transformant producing nitrile hydratase which has high activity also on aromatic nitriles.

The present invention relates to

(1) a $DNA^{(H)}$ fragment encoding a polypeptide having nitrile hydratase activity, said polypeptide comprising the $\alpha^{(H)}$-subunit as defined in the Sequence Listing by SEQ ID: No. 1 and the $\beta^{(H)}$-subunit as defined in the Sequence Listing by SEQ ID: No. 2;

(2) a $DNA^{(L)}$ fragment encoding a polypeptide having nitrile hydratase activity, said polypeptide comprising the $\alpha^{(L)}$-subunit as defined in the Sequence Listing by SEQ ID: No. 3 and the $\beta^{(L)}$-subunit as defined in the Sequence Listing by SEQ ID: No. 4;

(3) the $DNA^{(H)}$ fragment of (1) which contains a nucleotide sequence encoding said $\alpha^{(H)}$- and $\beta^{(H)}$-subunits, comprising the DNA sequence of the $\alpha^{(H)}$-subunit as defined in the Sequence Listing by SEQ ID: No. 5 and the DNA sequence of the $\beta^{(H)}$-subunit as defined in the Sequence Listing by SEQ ID: No. 6;

(4) the $DNA^{(L)}$ fragment of (2) which contains a nucleotide sequence encoding said $\alpha^{(L)}$- and $\beta^{(L)}$-subunits, comprising the DNA sequence of the $\alpha^{(L)}$-subunit as defined in the Sequence Listing by SEQ ID: No. 7 and the DNA sequence of the $\beta^{(L)}$subunit as defined in the Sequence Listing by SEQ ID: No. 8;

(5) a recombinant DNA comprising the $DNA^{(H)}$ or the $DNA^{(L)}$ of (1)-(4) in a vector;

(6) a transformant transformed with the recombinant DNA of (5);

(7) a method for the production of nitrile hydratase which comprises culturing the transformant as described in (6) and recovering nitrile hydratase from the culture;

(8) a method for the production of amides which comprises hydrating nitriles using nitrile hydratase as described in (7) to form amides; and

(9) a method for the production of amides which comprises culturing the transformant as described in (6), and hydrating nitriles using the resultant culture, isolated bacterial cells, treated matter thereof, or a fixed material of them, to form amides.

The present invention is described in detail as follows.

The present invention is carried out by the steps (1)-(8):

(1) Isolation and Purification of Nitrile Hydratase and Partial Amino Acid Sequencing of Nitrile Hydratase

Two types of nitrile hydratase (designated as H type and L type, respectively) are isolated and purified from Rhodococcus rhodochrous J-l (FERM BP-1478) and the both enzymes are separated into α and β subunits using HPLC. N-Terminal amino acid sequence each of the subunits is determined and shown in the Sequence Listing by SEQ ID: Nos. 9-12.

(2) Preparation of a DNA Probe for a Nitrile Hydratase Gene

A DNA probe is prepared from JM105/pYUK121 (FERM BP-1937) as described in JP-A-2-119778/1990 due to the high degree of homology in the amino acid sequence between the nitrile hydratase β subunit of Rhodococcus sp. N-774 described in said Japanese Patent Official Gazette and those of Rhodococcus rhodochrous J-I. Plasmid pYUK121 containing nitrile hydratase gene derived from Rhodococcus sp. N-774 is prepared from a JM105/pYUK121 culture. pYUK121 DNA is digested with SphI and SalI. The SphI-SalI fragment contains the nitrile hydratase gene (shown in the Sequence Listing by SEQ ID: No. 13) of Rhodococcus sp. N-774. The DNA fragment is radiolabeled.

(3) Detection of a DNA Segment Containing a Nitrile Hydratase Gene from the Chromosome ofRhodococcus rhodochrousJ-I

Chromosomal DNA is prepared from a Rhodococcus rhodochrous J-I culture. The chromosomal DNA is digested with restriction enzymes and hybridized to the probe described in (2) using the Southern hybridization method [Southern, E.M., J. Mol. Biol. 98, 503 (1975)].

Two DNA fragments of a different length are screened.

(4) Construction of a Recombinant Plasmid

A recombinant plasmid is constructed by inserting the chromosomal DNA fragment as prepared in (3) into a plasmid vector.

(5) Transformation and Screening for a Transformant Containing the Recombinant Plasmid

Transformants are prepared using the recombinant plasmid as described in (4). The transformant containing the recombinant plasmid is selected using the probe as described in (2) according to the colony hybridization method [R. Bruce Wallace et. al., Nuc. Aci. Res. 9, 879 (1981)]. Additionally, the presence of the nitrile hydratase gene in the recombinant plasmid is confirmed using the Southern hybridization method. The plasmids thus selected are designated as pNHJ10H and pNHJ20L.

(6) Isolation and Purification of Plasmid DNA and Construction of the Restriction Map

Plasmid DNAs of pNHJ10H and pNHJ20L as prepared in (5) are isolated and purified. The restriction map of the DNAs is constructed (Fig. 1) to determine the region containing nitrile hydratase gene.

(7) DNA Sequencing

The extra segment of the inserted DNA fragment in pNHJ10H and pNHJ20L is excised using an appropriate restriction enzyme. The inserted DNA fragment is then used for sequencing. The nucleotide sequence of the DNA fragment (SEQ: ID Nos. 14, 15) reveals that it contains the sequence deduced from the amino acid sequence as described in (1).

(8) Production of Nitrile Hydratase Using the Transformant and Conversion of Nitriles to Amides

The transformant as described in (8) is cultured. The bacterial cells are mixed with nitriles, a substrate of nitrile hydratase, and amides are produced.

Rhodococcus rhodochrous J-I was deposited with Fermentation Research Institute, Agency of Industrial Science and Technology, and was assigned the accession number FERM BP-1478. A transformant TGI/pNHJ10H containing pNHJ10H as described in (5) and a transformant TGI/pNHJ20L containing pNHJ20L as described in (5) were deposited with the above and assigned the accession number FERM BP-2777 and FERM BP-2778, respectively.

Any vectors including a plasmid vector (e.g., pAT153, pMP9, pHC624, pKC7, etc.), a phage vector (e.g., λgtII (Toyobo), Charon 4A (Amersham), etc.) may be used. Enzymes which may be used include SphI, SalI, EcoRI, BamHI, SacI, and the like, which are commercially available (Takara Shuzo). Various hosts may be used for transformation including but not limited to E. coli JM105 and E. coli TGI.

Culture media for the transformant are those ordinarily used in the art.

Conversion of nitriles to amides is carried out using nitrile hydratase, crude nitrile hydratase, the culture of the transformant, the isolated bacterial cells or treated matter thereof, and the like, prepared from the culture of the transformant.

Suitable nitriles in the invention include aromatic nitriles having 4-10 carbon atoms in the aromatic moiety and aliphatic nitriles having 2-6 carbon atoms, which are described in the European Patent Publication No. 0,307,926. Typical examples of the nitriles are 4-, 3- and 2-cyanopyridines, benzonitrile, 2,6-difluorobenzonitrile, 2-thiophene carbonitrile, 2-furonitrile, cyanopyrazine, acrylonitrile, methacrylonitrile, crotonitrile, acetonitrile and 3-hydroxypropionitrile.

The present invention discloses the amino acid sequence and nucleotide sequence of the α- and β-subunits of two types of nitrile hydratase derived from Rhodococcus rhodochrous J-l. The DNA fragment encoding nitrile hydratase is inserted into an expression vector and the recombinant vector is used for transformation. The transformant contains multiple copies of the gene and can produce much higher levels of nitrile hydratase than conventionally used microorganisms.

Fig. 1 shows restriction maps of recombinant plasmids, pNHJ10H and pNHJ20L.

The present invention is illustrated by the following Example.

The following abbreviations are used in the Example.

TE:	Tris-HCl (10 mM; pH 7.8), EDTA (1 mM, pH 8.0)
TNE:	Tris-HCl (50 mM; pH 8.0), EDTA (1 mM, pH 8.0), NaCl (50 mM)
STE:	Tris-HCl (50 mM; pk 8.0), EDTA (5 mM, pH 8.0), Sucrose (35 mM)
2xYT medium:	1.6% Trypton; 1.0% Yeast extract, 0.5% NaCl

Example

(1) Isolation and Purification of Nitrile Hydratase and Partial Amino Acid Sequencing of Nitrile Hydratase

Rhodococcus rhodochrous J-l was cultured in a medium (3 g/ℓ of yeast extract, 0.5 g/ℓ of KH_2PO_4, 0.5 g/ℓ of K_2HPO_4, 0.5 g/ℓ of $MgSO_4 \cdot 4H_2O$, 0.01 g/ℓ of $CoCl_2$, and 3 g/ℓ of crotonamide, pH 7.2) at 28°C for 80 hours. The bacterial cells were harvested. 50 g of the bacterial cells was disrupted and fractionated with ammonium sulfate. The sample was dialyzed and the dialysate was centrifuged. The supernatant was loaded on DEAE-Cellulofine chromatography, Phenyl-Sepharose chromatography, Sephadex G-150 chromatography and Octyl-Sepharose chromatography. Two fractions with enzyme activity were obtained and dialyzed. The dialysates were loaded on a high performance liquid chromatography using a reversed phase column (Senshu Pak VP-304-1251, Senshu Kagaku), and two respective subunits (α and β) were obtained. N-terminal amino acid sequence of $\alpha_1^{(H)}$-, $\beta_1^{(H)}$-, $\alpha_1^{(L)}$- and $\beta_1^{(L)}$-subunits was determined using an Applied Biosystems model 470A protein sequencer. The amino acid sequences are shown in the Sequence Listing by SEQ ID: Nos. 9-12.

(2) Preparation of a DNA Probe for Nitrile Hydratase Gene

E. coli JM105 (FERM BP-1937) containing pYUK121 was cultured in 100 ml of 2xYT medium containing 50 μg/ml of ampicillin at 30°C overnight (12 hours). The bacterial cells were harvested and TNE was added to the cells. The cell suspension was then centrifuged. 8 ml of STE and 10 mg of lysozyme were added to the pellet. The mixture was incubated at 0°C for five minutes followed by the addition of 4 ml of 0.25M EDTA. 2 ml of 10% SDS and 5 ml of 5M NaCl were then added to the mixture at room temperature. The resultant mixture was incubated at 0-4°C for three hours and then ultracentrifuged. 1/2 volume of 30% PEG 6000 was added to the supernatant. The mixture was incubated at 0-4°C overnight (12 hours) and centrifuged. TNE was added to the pellet to bring the volume to 7.5 ml and CsCl was then added to the suspension. The mixture was centrifuged to remove proteins. Then, 300-500 mg/ml of ethidium bromide was added to the supernatant. The mixture was transferred to a centrifuge tube. The tube was heat-sealed and then ultracentrifuged. cccDNA was extracted using a peristaltic pump. A bit more than equal amount of isopropyl alcohol saturated with water was added to the extract to rid of ethidium bromide. The sample was dialyzed against TE. About 3 ml of purified pYUK121 was obtained.

pYUK121 DNA was digested with SphI and SalI, resulting in a 2.07 kb DNA fragment containing a nitrile hydratase gene derived from Rhodococcus sp. N-774. The fragment was radiolabeled with ^{32}P to produce a probe. The nucleotide sequence of the probe is shown in the Sequence Listing by SEQ ID: No. 13.

(3) Preparation of a DNA Fragment Containing a Nitrile Hydratase Gene of Chromosome

Rhodococcus rhodochrous J-l was cultured in 100 ml of a medium (10 g/ℓ of glucose, 0.5 g/ℓ of KH_2PO_4, 0.5 g/ℓ of K_2HPO_4, 0.5 g/ℓ of $MgSO_4 \cdot 7H_2O$, 1 g/ℓ of yeast extract, 7.5 g/ℓ of peptone, 0.01 g/ℓ of $CoCl_2$, 7.5 g/ℓ of urea, 1% glycine or 0.2 μg/ml of ampicillin, 1 ℓ of water, pH 7.2). The bacterial cells

were harvested and the pellet was washed with TNE. The pellet was then suspended in 10 ml of TE. 4 ml of 0.25M EDTA, 10-20 mg of lysozyme, 10-20 mg of achromoprotease and 10 ml of 10×SDS were added to the suspension. The suspension was incubated at 37°C for three hours. 15 ml of phenol was added to the suspension. The mixture was incubated at room temperature for 15 minutes and then centrifuged. The upper layer was removed, and 0.7 ml of 2.5M sodium acetate and diethyl ether were added to the supernatant. The mixture was centrifuged and the upper layer was discarded. Two volumes of ethanol were added to the bottom layer and DNA was removed with a glass rod. DNA was rinsed for five minutes each with TE:ethanol 2:8, 1:9, and 0:10 (v/v). DNA was then resuspended in 2-4 ml of TE (37°C). 10 μℓ of a mixture of RNase A and T_1 was added to the suspension and the mixture was incubated at 37°C. An equal amount of phenol was added to the mixture which was then centrifuged. More than equal amount of ether was added to the supernatant. The mixture was centrifuged again, and the upper layer was discarded and the bottom layer was saved. The bottom layer was dialyzed against 2 ℓ of TE containing a small amount of chloroform overnight and further dialyzed against fresh TE for 3-4 hours. 4 ml of crude chromosomal DNA was obtained.

10 μℓ of TE, 3 μℓ of reaction buffer (10×) and 2 μℓ of SacI were added to 15 μℓ of crude chromosomal DNA. The mixture was incubated at 37°C for an hour and electrophoresed on an agarose gel at 60 V for three hours. The Southern hybridization of chromosomal DNA was carried out using the probe as described in (2). About 6.0 kb and 9.4 kb fragments were found to show a strong hybridization.

15 μℓ of chromosomal DNA was digested with SacI and electrophoresed on an agarose gel, as described above. 6.0 kb and 9.4 kb DNA fragments were cut out from the gel and taken in three volumes each of 8M $NaClO_4$. After solubilization, each solution was dotted on GF/C (Whatman) filter paper (6 mm in diameter). Ten drops (≈ 100 μℓ) of TE containing 6M $NaClO_4$ and then ten drops (≈ 100 μℓ) of 95% ethanol were added to the filter paper. The paper was air-dried for 3 minutes and placed in 0.5 ml Eppendorf tube. 40 μℓ of TE was added to the tube and the whole was incubated at 47°C for 30 minutes. The tube was then centrifuged. About 40 μℓ of the supernatant was obtained which contained 6.0 kb and 9.4 kb DNA fragments containing a nitrile hydratase gene of chromosomal DNA.

The method of inserting the 6.0 kb DNA fragment into a vector is described below. The same method is applied for the insertion of the 9.4 kb DNA fragment into a vector.

(4) Insertion of the Chromosomal DNA Fragment into a Vector

10 μℓ of TE, 3 μℓ of reaction buffer (10×) and 2 μℓ of SacI was added to 10 μℓ of pUC19. The mixture was incubated at 30°C for an hour. 2 μℓ of 0.25M EDTA was added to the mixture to stop the reaction. Then, 7 μℓ of 1m Tris-HCl (pH 9) and 3 μℓ of BAP (bacterial alkaline phosphatase) were added to the mixture. The mixture was incubated at 65°C for an hour. TE was then added to the mixture to make a total volume to 100 μℓ. The mixture was extracted 3× with an equal amount of phenol. An equal amount of ether was added to the extract. The bottom layer was removed and 10 μℓ of 3M sodium acetate and 250 μℓ of ethanol were added to the bottom layer. The mixture was incubated at -80°C for 30 minutes, centrifuged, dried, and resuspended in TE.

5 μℓ of pUC19 DNA thus obtained and 40 μℓ of the 6.0 kb DNA fragment as described in (3) were mixed. 6 μℓ of ligation buffer, 6 μℓ of ATP (6 mg/ml) and 3 μℓ of T4 DNA ligase were added to the mixture. The mixture was incubated at 4°C overnight (12 hours) to produce the recombinant plasmid containing the 6.0 kb DNA fragment encoding the desired enzyme in the SacI site of pUC19.

(5) Transformation and Screening of Transformants

E. coli TGI (Amersham) was inoculated into 10 ml of 2×YT medium and incubated at 37°C for 12 hours. After incubation, the resultant culture was added to fresh 2×YT medium to a concentration of 1%, and the mixture was incubated at 37°C for two hours. The culture was centrifuged and the pellet was suspended in 5 ml of cold 50 mM $CaCl_2$. The suspension was placed on ice for 40 minutes and then centrifuged. 0.25 ml of cold 50 mM $CaCl_2$ and 60 μℓ of the recombinant DNA as described in (4) were added to the pellet. The mixture was incubated at 0°C for 40 minutes, heat-shocked at 42°C for two minutes, placed on ice for five minutes, and added to 10 ml of 2×YT medium. The mixture was incubated at 37°C for 90 minutes with shaking, then centrifuged. The pellet was suspended in 1 ml of 2×YT medium, and two 10 μℓ aliquots of the suspension were plated on a 2×YT agar plate containing 50 μg/ml of ampicillin separately. The plate was incubated at 37°C. The colony grown on the plate was selected by the colony hybridization method: The colony was transferred to a nitrocellulose filter and digested. The DNA was fixed on the filter and hybridized to the probe as described in (2). The filter was autoradiographed and

a recombinant colony was selected. Additionally, the presence of a nitrile hydratase gene in the transformant was confirmed according to the Southern hybridization method.

(6) Isolation and Purification of Recombinant Plasmid and Construction of the Restriction Map of the Inserted DNA Fragments

The transformant selected as described in (5) was grown in 100 ml of 2×YT medium containing 50 μg/ml of ampicillin at 37°C overnight (12 hours). The bacterial cells were harvested and TNE was added to the cells. The cells were collected again by centrifugation, and 8 ml of STE and 10 mg of lysozyme were added to the cells. The mixture was incubated at 0°C for five minutes. 4 ml of 0.25M EDTA, 2 ml of 10% SDS (at room temperature) and 5 ml of 5M NaCl were added to the mixture. The mixture was incubated at 0-4°C for three hours, and ultracentrifuged. 1/2 volume of 30% PEG 6000 was added to the supernatant. The mixture was incubated at 0-4°C overnight (12 hours) and centrifuged again. TNE was added to the pellet to bring the volume up to 7.5 ml. CsCl was added to the suspension to rid of proteins. Then, 300-500 mg/ml of ethidium bromide was added to the supernatant and the mixture was transferred to a centrifuge tube. The tube was heat-sealed and ultracentrifuged. cccDNA was removed using a peristaltic pump. A bit more than equal amount of isopropyl alcohol saturated with water was added to cccDNA to remove ethidium bromide. The DNA sample was dialyzed against TE, resulting in about 3 ml of purified recombinant DNA. The recombinant plasmid thus obtained containing a 6.7 kb DNA fragment was designated as pNHJ10H (The recombinant plasmid containing a 9.4 kb DNA fragment was designated as pNHJ20L).

These plasmid DNAs were digested with EcoRI, BamHI, PstI, SacI and SalI. The restriction maps were constructed and are shown in Fig. 1.

(7) DNA Sequencing

The location of a nitrile hydratase gene in the DNA fragment of pNHJ10H was determined according to the restriction map constructed and to the Southern hybridization method. An extra segment in pNHJ10H was cleaved off with PstI and SalI: The 6.0 kb DNA fragment resulted in 1.97 kb. Similarly, an extra segment in pNHJ20L was cleaved off with EcoRI and SacI: The 9.4 kb DNA fragment resulted in 1.73 kb.

These DNA fragments were sequenced by the Sanger method [Sanger, F., Science 214: 1205-1210 (1981)] using M13 phage vector. The nucleotide sequence of the 1.97 kb DNA fragment (pNHJ10H) and the 1.73 kb DNA fragment (pNHJ20L) are shown in the Sequence Listing by SEQ ID: No. 14 and SEQ ID: No. 15, respectively.

The amino acid sequence deduced from the nucleotide sequence was found fully identical to the amino acid sequence as determined in (1). The sequence analysis also revealed that the DNA fragment contained the sequence coding for the α- and β-subunits.

(8) Production of Nitrile Hydratase Using the Transformant and Conversion of Nitriles to Amides Using Nitrile Hydratase

TG1 /pNHJ10H and TG1/pNHJ20L were inoculated into 10 ml of 2×YT medium containing 50 μg/ml of ampicillin and incubated at 30°C overnight (12 hours). 1 ml of the resultant culture was added to 100 ml of 2×YT medium (50 μg/ml of ampicillin, 0.1 g of $CoCl_2 \cdot 6H_2O/\ell$). The mixture was incubated at 30°C for 4 hours. IPTG was added to the mixture to a final concentration of 1 mM. The mixture was incubated at 30°C for 10 hours. After harvesting the cells, the cells were suspended in 5 ml of 0.1 M phosphate buffer (pH 7.5). The suspensions were disrupted by sonification for 5 min and centrifuged at 12,000 ×g for 30 min. The resulting supernatants were used for the enzyme assay. The enzyme assay was carried out in a reaction mixture (12 ml) containing 50 mM potassium phosphate buffer (pH 7.5), 6 mM benzonitrile and an appropriate amount of the enzyme. The reaction was carried out at 20°C for 30 min and stopped by the addition of 0.2 ml 1 M HCl. The amount of benzamide formed in the reaction mixture was determined by HPLC. As a control, the mixture obtained by the same procedure as described above but from E. coli TG1 was used. The levels of nitrile hydratase activity in cell-free extracts of E. coli containing pNHJ10H and pNHJ20L were 1.75 x 10^{-3} and 6.99 x 10^{-3} units/mg, respectively, when cultured in 2×YT medium in the presence of $CoCl_2$ and IPTG. Benzamide was found in the reaction mixture of TG1/pNHJ10H and pNHJ20L, whereas no benzamide was found in the reaction mixture of TG1 .

(1) INFORMATION FOR SEQ ID NO: 1

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 203 amino acids

(B) TYPE: Amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(vi) ORIGINAL SOURCE

(A) ORGANISM: Rhodococcus rhodochrous

(B) STRAIN: J-1 (FERM BP-1478)

(ix) FEATURES

(A) OTHER INFORMATION

$\alpha^{(H)}$-subunit

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1

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                5                  10                  15
MetSerGluHisValAsnLysTyrThrGluTyrGluAlaArgThr
                20                 25                  30
LysAlaIleGluThrLeuLeuTyrGluArgGlyLeuIleThrPro
                35                 40                  45
AlaAlaValAspArgValValSerTyrTyrGluAsnGluIleGly
                50                 55                  60
ProMetGlyGlyAlaLysValValAlaLysSerTrpValAspPro
                65                 70                  75
GluTyrArgLysTrpLeuGluGluAspAlaThrAlaAlaMetAla
                80                 85                  90
SerLeuGlyTyrAlaGlyGluGlnAlaHisGlnIleSerAlaVal
                95                100                 105
PheAsnAspSerGlnThrHisHisValValValCysThrLeuCys
               110                115                 120
SerCysTyrProTrpProValLeuGlyLeuProProAlaTrpTyr
               125                130                 135
LysSerMetGluTyrArgSerArgValValAlaAspProArgGly
```

140 145 150
ValLeuLysArgAspPheGlyPheAspIleProAspGluValGlu
155 160 165
ValArgValTrpAspSerSerSerGluIleArgTyrIleValIle
170 175 180
ProGluArgProAlaGlyThrAspGlyTrpSerGluGluGluLeu
185 190 195
ThrLysLeuValSerArgAspSerMetIleGlyValSerAsnAla
200
LeuThrProGlnGluValIleVal

(2) INFORMATION FOR SEQ ID NO: 2

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 229 amino acids

(B) TYPE: Amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(vi) ORIGINAL SOURCE

(A) ORGANISM: Rhodococcus rhodochrous

(B) STRAIN: J-1 (FERM BP-1478)

(ix) FEATURES

(A) OTHER INFORMATION

$\beta^{(H)}$-subunit

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2

5 10 15
MetAspGlyIleHisAspThrGlyGlyMetThrGlyTyrGlyPro
20 25 30
ValProTyrGlnLysAspGluProPhePheHisTyrGluTrpGlu
35 40 45
GlyArgThrLeuSerIleLeuThrTrpMetHisLeuLysGlyIle
50 55 60
SerTrpTrpAspLysSerArgPhePheArgGluSerMetGlyAsn
65 70 75
GluAsnTyrValAsnGluIleArgAsnSerTyrTyrThrHisTrp

80 85 90
LeuSerAlaAlaGluArgIleLeuValAlaAspLysIleIleThr
95 100 105
GluGluGluArgLysHisArgValGlnGluIleLeuGluGlyArg
110 115 120
TyrThrAspArgLysProSerArgLysPheAspProAlaGlnIle
125 130 135
GluLysAlaIleGluArgLeuHisGluProHisSerLeuAlaLeu
140 145 150
ProGlyAlaGluProSerPheSerLeuGlyAspLysIleLysVal
155 160 165
LysSerMetAsnProLeuGlyHisThrArgCysProLysTyrVal
170 175 180
ArgAsnLysIleGlyGluIleValAlaTyrHisGlyCysGlnIle
185 190 195
TyrProGluSerSerSerAlaGlyLeuGlyAspAspProArgPro
200 205 210
LeuTyrThrValAlaPheSerAlaGlnGluLeuTrpGlyAspAsp
215 220 225
GlyAsnGlyLysAspValValCysValAspLeuTrpGluProTyr

LeuIleSerAla

(3) INFORMATION FOR SEQ ID NO: 3

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 207 amino acids

(B) TYPE: Amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(vi) ORIGINAL SOURCE

(A) ORGANISM: Rhodococcus rhodochrous

(B) STRAIN: J-1 (FERM BP-1478)

(ix) FEATURES

(A) OTHER INFORMATION

$\alpha^{(L)}$-subunit

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3

```
                    5                   10                  15
MetThrAlaHisAsnProValGlnGlyThrLeuProArgSerAsn
                    20                  25                  30
GluGluIleAlaAlaArgValLysAlaMetGluAlaIleLeuVal
                    35                  40                  45
AspLysGlyLeuIleSerThrAspAlaIleAspHisMetSerSer
                    50                  55                  60
ValTyrGluAsnGluValGlyProGlnLeuGlyAlaLysIleVal
                    65                  70                  75
AlaArgAlaTrpValAspProGluPheLysGlnArgLeuLeuThr
                    80                  85                  90
AspAlaThrSerAlaCysArgGluMetGlyValGlyGlyMetGln
                    95                  100                 105
GlyGluGluMetValValLeuGluAsnThrGlyThrValHisAsn
                    110                 115                 120
MetValValCysThrLeuCysSerCysTyrProTrpProValLeu
                    125                 130                 135
GlyLeuProProAsnTrpTyrLysTyrProAlaTyrArgAlaArg
                    140                 145                 150
AlaValArgAspProArgGlyValLeuAlaGluPheGlyTyrThr
                    155                 160                 165
ProAspProAspValGluIleArgIleTrpAspSerSerAlaGlu
                    170                 175                 180
LeuArgTyrTrpValLeuProGlnArgProAlaGlyThrGluAsn
                    185                 190                 195
PheThrGluGluGlnLeuAlaAspLeuValThrArgAspSerLeu
                    200                 205
IleGlyValSerValProThrThrProSerLysAla
```

(4) INFORMATION FOR SEQ ID NO: 4

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 226 amino acids

(B) TYPE: Amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(vi) ORIGINAL SOURCE

(A) ORGANISM: Rhodococcus rhodochrous

(B) STRAIN: J-1 (FERM BP-1478)

(ix) FEATURES

(A) OTHER INFORMATION

$\beta^{(L)}$-subunit

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4

```
                 5                  10                  15
MetAspGlyIleHisAspLeuGlyGlyArgAlaGlyLeuGlyPro
                20                  25                  30
IleLysProGluSerAspGluProValPheHisSerAspTrpGlu
                35                  40                  45
ArgSerValLeuThrMetPheProAlaMetAlaLeuAlaGlyAla
                50                  55                  60
PheAsnLeuAspGlnPheArgGlyAlaMetGluGlnIleProPro
                65                  70                  75
HisAspTyrLeuThrSerGlnTyrTyrGluHisTrpMetHisAla
                80                  85                  90
MetIleHisHisGlyIleGluAlaGlyIlePheAspSerAspGlu
                95                 100                 105
LeuAspArgArgThrGlnTyrTyrMetAspHisProAspAspThr
               110                 115                 120
ThrProThrArgGlnAspProGlnLeuValGluThrIleSerGln
               125                 130                 135
LeuIleThrHisGlyAlaAspTyrArgArgProThrAspThrGlu
               140                 145                 150
AlaAlaPheAlaValGlyAspLysValIleValArgSerAspAla
               155                 160                 165
SerProAsnThrHisThrArgArgAlaGlyTyrValArgGlyArg
               170                 175                 180
ValGlyGluValValAlaThrHisGlyAlaTyrValPheProAsp
               185                 190                 195
ThrAsnAlaLeuGlyAlaGlyGluSerProGluHisLeuTyrThr
               200                 205                 210
ValArgPheSerAlaThrGluLeuTrpGlyGluProAlaAlaPro
               215                 220                 225
AsnValValAsnHisIleAspValPheGluProTyrLeuLeuPro

Ala
```

(5) INFORMATION FOR SEQ ID NO: 5

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 609 base pairs

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Genomic DNA

(vi) ORIGINAL SOURCE

(A) ORGANISM: Rhodococcus rhodochrous

(B) STRAIN: J-1 (FERM BP-1478)

(ix) FEATURES

(A) OTHER INFORMATION

$\alpha^{(H)}$-subunit

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5

```
             15             30             45
GTGAGCGAGCACGTCAATAAGTACACGGAGTACGAGGCACGTACC
             60             75             90
AAGGCGATCGAAACCTTGCTGTACGAGCGAGGGCTCATCACGCCC
            105            120            135
GCCGCGGTCGACCGAGTCGTTTCGTACTACGAGAACGAGATCGGC
            150            165            180
CCGATGGGCGGTGCCAAGGTCGTGGCCAAGTCCTGGGTGGACCCT
            195            210            225
GAGTACCGCAAGTGGCTCGAAGAGGACGCGACGGCCGCGATGGCG
            240            255            270
TCATTGGGCTATGCCGGTGAGCAGGCACACCAAATTTCGGCGGTC
            285            300            315
TTCAACGACTCCCAAACGCATCACGTGGTGGTGTGCACTCTGTGT
            330            345            360
TCGTGCTATCCGTGGCCGGTGCTTGGTCTCCCGCCCGCCTGGTAC
            375            390            405
AAGAGCATGGAGTACCGGTCCCGAGTGGTAGCGGACCCTCGTGGA
            420            435            450
GTGCTCAAGCGCGATTTCGGTTTCGACATCCCCGATGAGGTGGAG
```

```
              465            480            495
GTCAGGGTTTGGGACAGCAGCTCCGAAATCCGCTACATCGTCATC
              510            525            540
CCGGAACGGCCGGCCGGCACCGACGGTTGGTCCGAGGAGGAGCTG
              555            570            585
ACGAAGCTGGTGAGCCGGGACTCGATGATCGGTGTCAGTAATGCG
              600
CTCACACCGCAGGAAGTGATCGTA
```

(6) <u>INFORMATION FOR SEQ ID NO: 6</u>

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 687 nucleic acids

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Genomic DNA

(vi) ORIGINAL SOURCE

(A) ORGANISM: Rhodococcus rhodochrous

(B) STRAIN: J-1 (FERM BP-1478)

(ix) FEATURES

(A) OTHER INFORMATION

$\beta^{(H)}$-subunit

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6

```
               15             30             45
ATGGATGGTATCCACGACACAGGCGGCATGACCGGATACGGACCG
               60             75             90
GTCCCCTATCAGAAGGACGAGCCCTTCTTCCACTACGAGTGGGAG
              105            120            135
GGTCGGACCCTGTCAATTCTGACTTGGATGCATCTCAAGGGCATA
              150            165            180
TCGTGGTGGGACAAGTCGCGGTTCTTCCGGGAGTCGATGGGGAAC
              195            210            225
GAAAACTACGTCAACGAGATTCGCAACTCGTACTACACCCACTGG
              240            255            270
CTGAGTGCGGCAGAACGTATCCTCGTCGCCGACAAGATCATCACC
```

```
                     285                           300                           315
GAAGAAGAGCGAAAGCACCGTGTGCAAGAGATCCTTGAGGGTCGG
                     330                           345                           360
TACACGGACAGGAAGCCGTCGCGGAAGTTCGATCCGGCCCAGATC
                     375                           390                           405
GAGAAGGCGATCGAACGGCTTCACGAGCCCCACTCCCTAGCGCTT
                     420                           435                           450
CCAGGAGCGGAGCCGAGTTTCTCTCTCGGTGACAAGATCAAAGTG
                     465                           480                           495
AAGAGTATGAACCCGCTGGGACACACACGGTGCCCGAAATATGTG
                     510                           525                           540
CGGAACAAGATCGGGGAAATCGTCGCCTACCACGGCTGCCAGATC
                     555                           570                           585
TATCCCGAGAGCAGCTCCGCCGGCCTCGGCGACGATCCTCGCCCG
                     600                           615                           630
CTCTACACGGTCGCGTTTTCCGCCCAGGAACTGTGGGGCGACGAC
                     645                           660                           675
GGAAACGGGAAAGACGTAGTGTGCGTCGATCTCTGGGAACCGTAC

CTGATCTCTGCG
```

(7) <u>INFORMATION FOR SEQ ID NO: 7</u>

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 621 base pairs

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Genomic DNA

(vi) ORIGINAL SOURCE

(A) ORGANISM: Rhodococcus rhodochrous J-1 (FERM BP-1478)

(ix) FEATURES

(A) OTHER INFORMATION

$\alpha^{(L)}$-subunit

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7

```
            15             30             45
ATGACCGCCCACAATCCCGTCCAGGGCACGTTGCCACGATCGAAC
            60             75             90
GAGGAGATCGCCGCACGCGTGAAGGCCATGGAGGCCATCCTCGTC
           105            120            135
GACAAGGGCCTGATCTCCACCGACGCCATCGACCACATGTCCTCG
           150            165            180
GTCTACGAGAACGAGGTCGGTCCTCAACTCGGCGCCAAGATCGTC
           195            210            225
GCCCGCGCCTGGGTCGATCCCGAGTTCAAGCAGCGCCTGCTCACC
           240            255            270
GACGCCACCAGCGCCTGCCGTGAAATGGGCGTCGGCGGCATGCAG
           285            300            315
GGCGAAGAAATGGTCGTGCTGGAAAACACCGGCACGGTCCACAAC
           330            345            360
ATGGTCGTATGTACCTTGTGCTCGTGCTATCCGTGGCCGGTTCTC
           375            390            405
GGCCTGCCACCCAACTGGTACAAGTACCCCGCCTACCGCGCCCGC
           420            435            450
GCTGTCCGCGACCCCCGAGGTGTGCTGGCCGAATTCGGATATACC
           465            480            495
CCCGACCCTGACGTCGAGATCCGGATATGGGACTCGAGTGCCGAA
           510            525            540
CTTCGCTACTGGGTCCTGCCGCAACGCCCAGCCGGCACCGAGAAC
           555            570            585
TTCACCGAAGAACAACTCGCCGACCTCGTCACCCGCGACTCGCTC
           600            615
ATCGGCGTATCCGTCCCCACCACACCCAGCAAGGCC
```

(8) INFORMATION FOR SEQ ID NO: 8

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 678 base pairs

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Genomic DNA

(vi) ORIGINAL SOURCE

(A) ORGANISM: Rhodococcus rhodochrous

(B) STRAIN: J-1 (FERM BP-1478)

(ix) FEATURES

(A) OTHER INFORMATION

$\beta^{(L)}$-subunit

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8

```
             15             30             45
ATGGATGGAATCCACGACCTCGGTGGCCGCGCCGGCCTGGGTCCG
             60             75             90
ATCAAGCCCGAATCCGATGAACCTGTTTTCCATTCCGATTGGGAG
            105            120            135
CGGTCGGTTTTGACGATGTTCCCGGCGATGGCGCTGGCCGGCGCG
            150            165            180
TTCAATCTCGACCAGTTCCGGGGCGCGATGGAGCAGATCCCCCCG
            195            210            225
CACGACTACCTGACCTCGCAATACTACGAGCACTGGATGCACGCG
            240            255            270
ATGATCCACCACGGCATCGAGGCGGGCATCTTCGATTCCGACGAA
            285            300            315
CTCGACCGCCGCACCCAGTACTACATGGACCATCCGGACGACACG
            330            345            360
ACCCCCACGCGGCAGGATCCGCAACTGGTGGAGACGATCTCGCAA
            375            390            405
CTGATCACCCACGGAGCCGATTACCGACGCCCGACCGACACCGAG
            420            435            450
GCCGCATTCGCCGTAGGCGACAAAGTCATCGTGCGGTCGGACGCC
            465            480            495
TCACCGAACACCCACACCCGCCGCGCCGGATACGTCCGCGGTCGT
            510            525            540
GTCGGCGAAGTCGTGGCGACCCACGGCGCGTATGTCTTTCCGGAC
            555            570            585
ACCAACGCACTCGGCGCCGGCGAAAGCCCCGAACACCTGTACACC
            600            615            630
GTGCGGTTCTCGGCGACCGAGTTGTGGGGTGAACCTGCCGCCCCG
            645            660            675
AACGTCGTCAATCACATCGACGTGTTCGAACCGTATCTGCTACCG

GCC
```

(9) INFORMATION FOR SEQ ID NO: 9

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 amino acids

(B) TYPE: Amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(vi) ORIGINAL SOURCE

(A) ORGANISM: Rhodococcus rhodochrous

(B) STRAIN: J-1 (FERM BP-1478)

(ix) FEATURES

(A) OTHER INFORMATION

$\alpha^{(H)}$-subunit: $\alpha_1^{(H)}$

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9

```
                        5                       10                      15
Ser-Glu-His-Val-Asn-Lys-Tyr-Thr-Glu-Tyr-Glu-Ala-Arg-Thr-Lys
                20                      25
Ala-Ile-Glu-Thr-Leu-Leu-Tyr-Glu-Arg-Gly-Leu
```

(10) <u>INFORMATION FOR SEQ ID NO: 10</u>

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 amino acids

(B) TYPE: Amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(vi) ORIGINAL SOURCE

(A) ORGANISM: Rhodococcus rhodochrous

(B) STRAIN: J-1 (FERM BP-1478)

(ix) FEATURES

(A) OTHER INFORMATION

$\beta^{(H)}$-subunit: $\beta_1^{(H)}$

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10

```
                    5                        10                        15
Met-Asp-Gly-Ile-His-Asp-Thr-Gly-Gly-Met-Thr-Gly-Tyr-Gly-Pro
                   20                        25
Val-Pro-Tyr-Gln-Lys-Asp-Glu-Pro-Phe-Phe-His-Tyr-Glu
```

(11) <u>INFORMATION FOR SEQ ID NO: 11</u>

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids

(B) TYPE: Amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(vi) ORIGINAL SOURCE

(A) ORGANISM: Rhodococcus rhodochrous

(B) STRAIN: J-1 (FERM BP-1478)

(ix) FEATURES

(A) OTHER INFORMATION

$\alpha^{(L)}$-subunit: $\alpha_1^{(L)}$

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11

5 10 15

Thr-Ala-His-Asn-Pro-Val-Gln-Gly-Thr-Leu-Pro-Arg-?-Asn-Glu

(12) INFORMATION FOR SEQ ID NO: 12

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 amino acids

(B) TYPE: Amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(vi) ORIGINAL SOURCE

(A) ORGANISM: Rhodococcus rhodochrous

(B) STRAIN: J-1 (FERM BP-1478)

(ix) FEATURES

(A) OTHER INFORMATION

$\beta^{(L)}$-subunit: $\beta_1{}^{(L)}$

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12

5 10 15

Met-Asp-Gly-Ile-His-Asp-Leu-Gly-Gly-Arg-Ala-?-Leu-?-Pro

Ile-Lys-Pro-Glu

(13) INFORMATION FOR SEQ ID NO: 13

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2070 base pairs

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Genomic DNA

(vi) ORIGINAL SOURCE

(A) ORGANISM: Rhodococcus sp.

(B) STRAIN: N-774 (FERM BP-1936)

(ix) FEATURES

from nucleotide No. 675 to 1295: subunit α

from nucleotide No. 1225 to 1960: subunit β

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13

```
SphI
GCATGCTTTCCACATCTGGAACGTGATCGCCACGGACGGTGGTG
  50
CCTACCAGATGTTGGACGGCAACGGATACGGCATGAACGCCGAAG
  100
GTTTGTACGATCCGGAACTGATGGCACACTTTGCTTCTCGACGCA
  150
TTCAGCACGCCGACGCTCTGTCCGAAACCGTCAAACTGGTGGCCC
  200
TGACCGGCCACCACGGCATCACCACCCTCGGCGGCGCGAGCTACG
  250
GCAAAGCCCGGAACCTCGTACCGCTTGCCCGCGCCGCCTACGACA
  300
CTGCCTTGAGACAATTCGACGTCCTGGTGATGCCAACGCTGCCCT
  350
ACGTCGCATCCGAATTGCCGGCGAAGGACGTAGATCGTGCAACCT
```

```
                                             .400
TCATCACCAAGGCTCTCGGGATGATCGCCAACACGGCACCATTCG

ACGTGACCGGACATCCGTCCCTGTCCGTTCCGGCCGGCCTGGTGA
.450
ACGGGGTTCCGGTCGGAATGATGATCACCGGCAGACACTTCGACG
    .500                                HindⅢ
ATGCGACAGTCCTTCGTGTCGGACGCGCATTCGAAAAGCTTCGCG
           .550
GCCGGTTTCCGACGCCGGCCGAACGCGCCTCCAACTCTGCACCAC
                   .600
AACTCAGCCCCGCCTAGTCCTGACGCACTGTCAGACAACAAATTC
                              .650
CACCGATTCACACATGATCAGCCCACATAAGAAAAGGTGAACCAG
                                   .700
ATGTCAGTAACGATCGACCACACAACGGAGAACGCCGCACCGGCC
MetSerValThrIleAspHisThrThrGluAsnAlaAlaProAla
  Subunitα                          .750
CAGGCGGCGGTCTCCGACCGGGCGTGGGCACTGTTCCGCGCACTC
GlnAlaAlaValSerAspArgAlaTrpAlaLeuPheArgAlaLeu
                 KpnI                    .800
GACGGTAAGGGATTGGTACCCGACGGTTACGTCGAGGGATGGAAG
AspGlyLysGlyLeuValProAspGlyTyrValGluGlyTrpLys
                                             .850
AAGACCTCCGAGGAGGACTTCAGTCCAAGGCGCGGAGCGGAATTG
LysThrSerGluGluAspPheSerProArgArgGlyAlaGluLeu
                                       PvuⅡ
GTAGCGCGCGCATGGACCGACCCCGAGTTCCGGCAGCTGCTTCTC
ValAlaArgAlaTrpThrAspProGluPheArgGlnLeuLeuLeu
.900   KpnI
ACCGACGGTACCGCCGCAGTTGCCCAGTACGGATACCTGGGCCCC
ThrAspGlyThrAlaAlaValAlaGlnTyrGlyTyrLeuGlyPro
    .950
CAGGCGGCCTACATCGTGGCAGTCGAAGACACCCCGACACTCAAG
GlnAlaAlaTyrIleValAlaValGluAspThrProThrLeuLys
          .1000
AACGTGATCGTGTGCTCGCTGTGTTCATGCACCGCGTGGCCCATC
AsnValIleValCysSerLeuCysSerCysThrAlaTrpProIle
                   .1050
CTCGGTCTGCCACCCACCTGGTACAAGAGCTTCGAATACCGTGCG
LeuGlyLeuProProThrTrpTyrLysSerPheGluTyrArgAla
```

```
                         .1100
CGCGTGGTCCGCGAACCACGGAAGGTTCTCTCCGAGATGGGAACC
ArgValValArgGluProArgLysValLeuSerGluMetGlyThr

                                     .1150
GAGATCGCGTCGGACATCGAGATTCGCGTCTACGACACCACCGCC
GluIleAlaSerAspIleGluIleArgValTyrAspThrThrAla

                                           .1200
GAAACTCGCTACATGGTCCTCCCGCAGCGTCCCGCCGGCACCGAA
GluThrArgTyrMetValLeuProGlnArgProAlaGlyThrGlu
                         PstI             .1250
GGCTGGAGCCAGGAACAACTGCAGGAAATCGTCACCAAGGACTGC
GlyTrpSerGlnGluGlnLeuGlnGluIleValThrLysAspCys

                                               .1300
CTGATCGGGGTTGCAATCCCGCAGGTTCCCACCGTCTGATCACCC
LeuIleGlyValAlaIleProGlnValProThrValTRM

CGACAAGAAGGAAGCACACC-ATGGATGGAGTACACGATCTTGCC
                     MetAspGlyValHisAspLeuAla
                     Subunit β
 .1350
GGAGTACAAGGCTTCGGCAAAGTCCCGCATACCGTCAACGCCGAC
GlyValGlnGlyPheGlyLysValProHisThrValAsnAlaAsp
        .1400
ATCGGCCCCACCTTTCACGCCGAATGGGAACACCTGCCCTACAGC
IleGlyProThrPheHisAlaGluTrpGluHisLeuProTyrSer
          .1450                              SalI
CTGATGTTCGCCGGTGTCGCCGAACTCGGGGCCTTCAGCGTCGAC
LeuMetPheAlaGlyValAlaGluLeuGlyAlaPheSerValAsp
                 .1500
GAAGTGCGATACGTCGTCGAGCGGATGGAGCCGGGCCACTACATG
GluValArgTyrValValGluArgMetGluProGlyHisTyrMet
                         .1550
ATGACCCCGTACTACGAGAGGTACGTCATCGGTGTCGCGACATTG
MetThrProTyrTyrGluArgTyrValIleGlyValAlaThrLeu
                               .1600
ATGGTCGAAAAGGGAATCCTGACGCAGGACGAACTCGAAAGCCTT
MetValGluLysGlyIleLeuThrGlnAspGluLeuGluSerLeu
                                       .1650
GCGGGGGGACCGTTCCCACTGTCACGGCCCAGCGAATCCGAAGGG
AlaGlyGlyProPheProLeuSerArgProSerGluSerGluGly
```

```
                                             .1700
CGGCCGGCACCCGTCGAGACGACCACCTTCGAAGTCGGGCAGCGA
ArgProAlaProValGluThrThrThrPheGluValGlyGlnArg

                                                 .1750
GTACGCGTACGCGACGAGTACGTTCCGGGGCATATTCGAATGCCT
ValArgValArgAspGluTyrValProGlyHisIleArgMetPro

GCATACTGCCGTGGACGAGTGGGAACCATCTCTCATCGAACTACC
AlaTyrCysArgGlyArgValGlyThrIleSerHisArgThrThr
 .1800
GAGAAGTGGCCGTTTCCCGACGCAATCGGCCACGGGCGCAACGAC
GluLysTrpProPheProAspAlaIleGlyHisGlyArgAsnAsp
      .1850
GCCGGCGAAGAACCGACGTACCACGTGAAGTTCGCCGCCGAGGAA
AlaGlyGluGluProThrTyrHisValLysPheAlaAlaGluGlu
           .1900                        SalI
TTGTTCGGTAGCGACACCGACGGTGGAAGCGTCGTTGTCGACCTC
LeuPheGlySerAspThrAspGlyGlySerValValValAspLeu
                .1950
TTCGAGGGTTACCTCGAGCCTGCGGCCTGATCTTCCAGCATTCCA
PheGluGlyTyrLeuGluProAlaAlaTRM
                     .2000
GGCGGCGGTCACGCGATCACAGCGGTTCGTGCGACCGCCGCCTGA
                              .2050
TCACCACGATTCACTCATTCGGAAGGACACTGGAAATCATGGTCG
SalI
AC
```

(14) <u>INFORMATION FOR SEQ ID NO: 14</u>

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1970 base pairs

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Genomic DNA

(vi) ORIGINAL SOURCE

(A) ORGANISM: Rhodococcus rhodochrous J-1

(FERM BP-1478)

(ix) FEATURES

from nucleotide No. 408 to 1094: subunit $\beta^{(H)}$

from nucleotide No. 1111 to 1719: subunit $\alpha^{(H)}$

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14

```
         10         20         30         40         50         60
CTGCAGCTCGAACATCGAAGGGTGCGAGCCGAGAGATCGGAGACGCAGACACCCGGAGGG

         70         80         90        100        110        120
AACTTAGCCTCCCGGACCGATGCGTGTCCTGGCAACGCCTCAAAATTCAGTGCAAGCGAT

        130        140        150        160        170        180
TCAATCTTGTTACTTCCAGAACCGAATCACGTCCCCGTAGTGTGCGGGGAGAGCGCCCGA

        190        200        210        220        230        240
ACGCAGGGATGGTATCCATGCGCCCCTTCTCTTTTCGAACGAGAACCGGCCGGTACAGCC

        250        260        270        280        290        300
GACCCGGAGACACTGTGACGCCGTTCAACGATTGTTGTGCTGTGAAGGATTCACCCAAGC

        310        320        330        340        350        360
CAACTGATATCGCCATTCCGTTGCCGGAACATTTGACACCTTCTCCCTACGAGTAGAAGC

        370        380        390        400        410        420
CAGCTGGACCCCTCTTTGAGCCCAGCTCCGATGAAAGCAATGAGGAAATGGATGGTATCC
                                                  MetAspGlyIleH
                                                  Subunit β(H)

        430        440        450        460        470        480
ACGACACAGGCGGCATGACCGGATACGGACCGGTCCCCTATCAGAAGGACGAGCCCTTCT
isAspThrGlyGlyMetThrGlyTyrGlyProValProTyrGlnLysAspGluProPheP

        490        500        510        520        530        540
TCCACTACGAGTGGGAGGGTCGGACCCTGTCAATTCTGACTTGGATGCATCTCAAGGGCA
heHisTyrGluTrpGluGlyArgThrLeuSerIleLeuThrTrpMetHisLeuLysGlyI

        550        560        570        580        590        600
TATCGTGGTGGGACAAGTCGCGGTTCTTCCGGGAGTCGATGGGGAACGAAAACTACGTCA
leSerTrpTrpAspLysSerArgPhePheArgGluSerMetGlyAsnGluAsnTyrValA

        610        620        630        640        650        660
ACGAGATTCGCAACTCGTACTACACCCACTGGCTGAGTGCGGCAGAACGTATCCTCGTCG
snGluIleArgAsnSerTyrTyrThrHisTrpLeuSerAlaAlaGluArgIleLeuValA

        670        680        690        700        710        720
CCGACAAGATCATCACCGAAGAAGAGCGAAAGCACCGTGTGCAAGAGATCCTTGAGGGTC
laAspLysIleIleThrGluGluGluArgLysHisArgValGlnGluIleLeuGluGlyA
```

```
      730        740        750        760        770        780
GGTACACGGACAGGAAGCCGTCGCGGAAGTTCGATCCGGCCCAGATCGAGAAGGCGATCG
rgTyrThrAspArgLysProSerArgLysPheAspProAlaGlnIleGluLysAlaIleG

      790        800        810        820        830        840
AACGGCTTCACGAGCCCCACTCCCTAGCGCTTCCAGGAGCGGAGCCGAGTTTCTCTCTCG
luArgLeuHisGluProHisSerLeuAlaLeuProGlyAlaGluProSerPheSerLeuG

      850        860        870        880        890        900
GTGACAAGATCAAAGTGAAGAGTATGAACCCGCTGGGACACACACGGTGCCCGAAATATG
lyAspLysIleLysValLysSerMetAsnProLeuGlyHisThrArgCysProLysTyrV

      910        920        930        940        950        960
TGCGGAACAAGATCGGGGAAATCGTCGCCTACCACGGCTGCCAGATCTATCCCGAGAGCA
alArgAsnLysIleGlyGluIleValAlaTyrHisGlyCysGlnIleTyrProGluSerS

      970        980        990       1000       1010       1020
GCTCCGCCGGCCTCGGCGACGATCCTCGCCCGCTCTACACGGTCGCGTTTTCCGCCCAGG
erSerAlaGlyLeuGlyAspAspProArgProLeuTyrThrValAlaPheSerAlaGlnG

     1030       1040       1050       1060       1070       1080
AACTGTGGGGCGACGACGGAAACGGGAAAGACGTAGTGTGCGTCGATCTCTGGGAACCGT
luLeuTrpGlyAspAspGlyAsnGlyLysAspValValCysValAspLeuTrpGluProT

     1090       1100       1110       1120       1130       1140
ACCTGATCTCTGCGTGAAAGGAATACGATAGTGAGCGAGCACGTCAATAAGTACACGGAG
yrLeuIleSerAla                    MetSerGluHisValAsnLysTyrThrGlu
                                  Subunit α (H)

     1150       1160       1170       1180       1190       1200
TACGAGGCACGTACCAAGGCGATCGAAACCTTGCTGTACGAGCGAGGGCTCATCACGCCC
TyrGluAlaArgThrLysAlaIleGluThrLeuLeuTyrGluArgGlyLeuIleThrPro

     1210       1220      11230       1240       1250       1260
GCCGCGGTCGACCGAGTCGTTTCGTACTACGAGAACGAGATCGGCCCGATGGGCGGTGCC
AlaAlaValAspArgValValSerTyrTyrGluAsnGluIleGlyProMetGlyGlyAla

     1270       1280       1290       1300       1310       1320
AAGGTCGTGGCCAAGTCCTGGGTGGACCCTGAGTACCGCAAGTGGCTCGAAGAGGACGCG
LysValValAlaLysSerTrpValAspProGluTyrArgLysTrpLeuGluGluAspAla

     1330       1340       1350       1360       1370       1380
ACGGCCGCGATGGCGTCATTGGGCTATGCCGGTGAGCAGGCACACCAAATTTCGGCGGTC
ThrAlaAlaMetAlaSerLeuGlyTyrAlaGlyGluGlnAlaHisGlnIleSerAlaVal

     1390       1400       1410       1420       1430       1440
TTCAACGACTCCCAAACGCATCACGTGGTGGTGTGCACTCTGTGTTCGTGCTATCCGTGG
PheAsnAspSerGlnThrHisHisValValValCysThrLeuCysSerCysTyrProTrp
```

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        1450              1460              1470              1480              1490              1500
CCGGTGCTTGGTCTCCCGCCCGCCTGGTACAAGAGCATGGAGTACCGGTCCCGAGTGGTA
ProValLeuGlyLeuProProAlaTrpTyrLysSerMetGluTyrArgSerArgValVal

        1510              1520              1530              1540              1550              1560
GCGGACCCTCGTGGAGTGCTCAAGCGCGATTTCGGTTTCGACATCCCCGATGAGGTGGAG
AlaAspProArgGlyValLeuLysArgAspPheGlyPheAspIleProAspGluValGlu

        1570              1580              1590              1600              1610              1620
GTCAGGGTTTGGGACAGCAGCTCCGAAATCCGCTACATCGTCATCCCGGAACGGCCGGCC
ValArgValTrpAspSerSerSerGluIleArgTyrIleValIleProGluArgProAla

        1630              1640              1650              1660              1670              1680
GGCACCGACGGTTGGTCCGAGGAGGAGCTGACGAAGCTGGTGAGCCGGGACTCGATGATC
GlyThrAspGlyTrpSerGluGluGluLeuThrLysLeuValSerArgAspSerMetIle

        1690              1700              1710              1720              1730              1740
GGTGTCAGTAATGCGCTCACACCGCAGGAAGTGATCGTATGAGTGAAGACACACTCACTG
GlyValSerAsnAlaLeuThrProGlnGluValIleVal

        1750              1760              1770              1780              1790              1800
ATCGGCTCCCGGCGACTGGGACCGCCGCACCGCCCCGCGACAATGGCGAGCTTGTATTCA

        1810              1820              1830              1840              1850              1860
CCGAGCCTTGGGAAGCAACGGCATTCGGGGTCGCCATCGCGCTTTCGGATCAGAAGTCGT

        1870              1880              1890              1900              1910              1920
ACGAATGGGAGTTCTTCCGACAGCGTCTCATTCACTCCATCGCTGAGGCCAACGGTTGCG

        1930              1940              1950              1960              1970
AGGCATACTACGAGAGCTGGACAAAGGCGCTCGAGGCCAGCGTGGTCGAC
```

(15) <u>INFORMATION FOR SEQ ID NO: 15</u>

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1731 base pairs

(B) TYPE: Nucleic acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Genomic DNA

(vi) ORIGINAL SOURCE

(A) ORGANISM: Rhodococcus rhodochrous

(B) STRAIN: J-1 (FERM BP-1478)

(ix) FEATURES

from nucleotide No. 171 to 848: subunit $\beta^{(L)}$

from nucleotide No. 915 to 1535: subunit $\alpha^{(L)}$

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15

```
        10        20        30        40        50        60
GAGCTCCCTGGAGCCACTCGCGCCGACGCATCCACGCTCGGACAGCCCACGGTGCGGATC

        70        80        90       100       110       120
ACCCCTGTTCGTCGGTAACAGAACAGTAACATGTCATCAGGTCATGACGTGTTGACGCAT

       130       140       150       160       170       180
TAGACGAGGGCACATAGGGTTGGTGACTCACGGCACAAGGAGAGCATTTCATGGATGGAA
                                                     MetAspGlyI
                                                     Subunit β(L)

       190       200       210       220       230       240
TCCACGACCTCGGTGGCCGCGCCGGCCTGGGTCCGATCAAGCCCGAATCCGATGAACCTG
leHisAspLeuGlyGlyArgAlaGlyLeuGlyProIleLysProGluSerAspGluProV

       250       260       270       280       290       300
TTTTCCATTCCGATTGGGAGCGGTCGGTTTTGACGATGTTCCCGGCGATGGCGCTGGCCG
alPheHisSerAspTrpGluArgSerValLeuThrMetPheProAlaMetAlaLeuAlaG

       310       320       330       340       350       360
GCGCGTTCAATCTCGACCAGTTCCGGGCGCGATGGAGCAGATCCCCCCGCACGACTACC
lyAlaPheAsnLeuAspGlnPheArgGlyAlaMetGluGlnIleProProHisAspTyrL

       370       380       390       400       410       420
TGACCTCGCAATACTACGAGCACTGGATGCACGCGATGATCCACCACGGCATCGAGGCGG
euThrSerGlnTyrTyrGluHisTrpMetHisAlaMetIleHisHisGlyIleGluAlaG

       430       440       450       460       470       480
GCATCTTCGATTCCGACGAACTCGACCGCCGCACCCAGTACTACATGGACCATCCGGACG
lyIlePheAspSerAspGluLeuAspArgArgThrGlnTyrTyrMetAspHisProAspA
```

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      490       500       510       520       530       540
ACACGACCCCCACGCGGCAGGATCCGCAACTGGTGGAGACGATCTCGCAACTGATCACCC
spThrThrProThrArgGlnAspProGlnLeuValGluThrIleSerGlnLeuIleThrH

      550       560       570       580       590       600
ACGGAGCCGATTACCGACGCCCGACCGACACCGAGGCCGCATTCGCCGTAGGCGACAAAG
isGlyAlaAspTyrArgArgProThrAspThrGluAlaAlaPheAlaValGlyAspLysV

      610       620       630       640       650       660
TCATCGTGCGGTCGGACGCCTCACCGAACACCCACACCCGCCGCGCCGGATACGTCCGCG
alIleValArgSerAspAlaSerProAsnThrHisThrArgArgAlaGlyTyrValArgG

      670       680       690       700       710       720
GTCGTGTCGGCGAAGTCGTGGCGACCCACGGCGCGTATGTCTTTCCGGACACCAACGCAC
lyArgValGlyGluValValAlaThrHisGlyAlaTyrValPheProAspThrAsnAlaL

      730       740       750       760       770       780
TCGGCGCCGGCGAAAGCCCCGAACACCTGTACACCGTGCGGTTCTCGGCGACCGAGTTGT
euGlyAlaGlyGluSerProGluHisLeuTyrThrValArgPheSerAlaThrGluLeuT

      790       800       810       820       830       840
GGGGTGAACCTGCCGCCCCGAACGTCGTCAATCACATCGACGTGTTCGAACCGTATCTGC
rpGlyGluProAlaAlaProAsnValValAsnHisIleAspValPheGluProTyrLeuL

      850       860       870       880       890       900
TACCGGCCTGACCAGGTCATCCGGTCCACCCAGCGAGACGTCCCTTCACCACAGACAGAA
euProAla

      910       920       930       940       950       960
ACGAGCCCACCCCGATGACCGCCCACAATCCCGTCCAGGGCACGTTGCCACGATCGAACG
                  MetThrAlaHisAsnProValGlnGlyThrLeuProArgSerAsnG
                  Subunit α (L)

      970       980       990      1000      1010      1020
AGGAGATCGCCGCACGCGTGAAGGCCATGGAGGCCATCCTCGTCGACAAGGGCCTGATCT
luGluIleAlaAlaArgValLysAlaMetGluAlaIleLeuValAspLysGlyLeuIleS

     1030      1040      1050      1060      1070      1080
CCACCGACGCCATCGACCACATGTCCTCGGTCTACGAGAACGAGGTCGGTCCTCAACTCG
erThrAspAlaIleAspHisMetSerSerValTyrGluAsnGluValGlyProGlnLeuG

     1090      1100      1110      1120      1130      1140
GCGCCAAGATCGTCGCCCGCGCCTGGGTCGATCCCGAGTTCAAGCAGCGCCTGCTCACCG
lyAlaLysIleValAlaArgAlaTrpValAspProGluPheLysGlnArgLeuLeuThrA

     1150      1160      1170      1180      1190      1200
ACGCCACCAGCGCCTGCCGTGAAATGGGCGTCGGCGGCATGCAGGGCGAAGAAATGGTCG
spAlaThrSerAlaCysArgGluMetGlyValGlyGlyMetGlnGlyGluGluMetValV

     1210      1220      1230      1240      1250      1260
TGCTGGAAAACACCGGCACGGTCCACAACATGGTCGTATGTACCTTGTGCTCGTGCTATC
alLeuGluAsnThrGlyThrValHisAsnMetValValCysThrLeuCysSerCysTyrP

     1270      1280      1290      1300      1310      1320
CGTGGCCGGTTCTCGGCCTGCCACCCAACTGGTACAAGTACCCCGCCTACCGCGCCCGCG
roTrpProValLeuGlyLeuProProAsnTrpTyrLysTyrProAlaTyrArgAlaArgA

     1330      1340      1350      1360      1370      1380
CTGTCCGCGACCCCCGAGGTGTGCTGGCCGAATTCGGATATACCCCCGACCCTGACGTCG
laValArgAspProArgGlyValLeuAlaGluPheGlyTyrThrProAspProAspValG
```

```
         1390          1400          1410          1420          1430          1440
AGATCCGGATATGGGACTCGAGTGCCGAACTTCGCTACTGGGTCCTGCCGCAACGCCCAG
luIleArgIleTrpAspSerSerAlaGluLeuArgTyrTrpValLeuProGlnArgProA
         1450          1460          1470          1480          1490          1500
CCGGCACCGAGAACTTCACCGAAGAACAACTCGCCGACCTCGTCACCCGCGACTCGCTCA
laGlyThrGluAsnPheThrGluGluGlnLeuAlaAspLeuValThrArgAspSerLeuI
         1510          1520          1530          1540          1550          1560
TCGGCGTATCCGTCCCCACCACACCCAGCAAGGCCTGACATGCCCCGACTCAACGAACAA
leGlyValSerValProThrThrProSerLysAla
         1570          1580          1590          1600          1610          1620
CCCCACCCGGGTCTCGAAGCCAACCTCGGCGACCTGGTACAGAATCTGCCGTTCAACGAA

         1630          1640          1650          1660          1670          1680
CGAATCCCCCGCCGCTCCGGCGAGGTCGCCTTCGATCAGGCCTGGGAGATCCGCGCCTTC

         1690          1700          1710          1720          1730
AGCATTGCCACCGCATTGCATGGCCAGGGCCGATTCGAATGGGACGAATTC
```

Claims

1. A DNA$^{(H)}$ fragment encoding a polypeptide having nitrile hydratase activity, said polypeptide comprising the $\alpha^{(H)}$-subunit as defined in the Sequence Listing by SEQ ID: No. 1 and the $\beta^{(H)}$-subunit as defined in the Sequence Listing by SEQ ID: No. 2.

2. A DNA$^{(L)}$ fragment encoding a polypeptide having nitrile hydratase activity, said polypeptide comprising the $\alpha^{(L)}$-subunit as defined in the Sequence Listing by SEQ ID: No. 3 and the $\beta^{(L)}$-subunit as defined in the Sequence Listing by SEQ ID: No. 4.

3. The DNA$^{(H)}$ fragment of claim 1 which contains the nucleotide sequences of the $\alpha^{(H)}$-subunit as defined in the Sequence Listing by SEQ ID: No. 5 and the $\beta^{(H)}$-subunit as defined in the Sequence Listing by SEQ ID: No. 6.

4. The DNA$^{(L)}$ fragment of claim 2 which contains the nucleotide sequences of the $\alpha^{(L)}$-subunit as defined in the Sequence Listing by SEQ ID: No. 7 and the $\beta^{(L)}$-subunit as defined in the Sequence Listing by SEQ ID: No. 8.

5. A recombinant DNA comprising a DNA$^{(H)}$ or DNA$^{(L)}$ of any one of claims 1-4 in a vector.

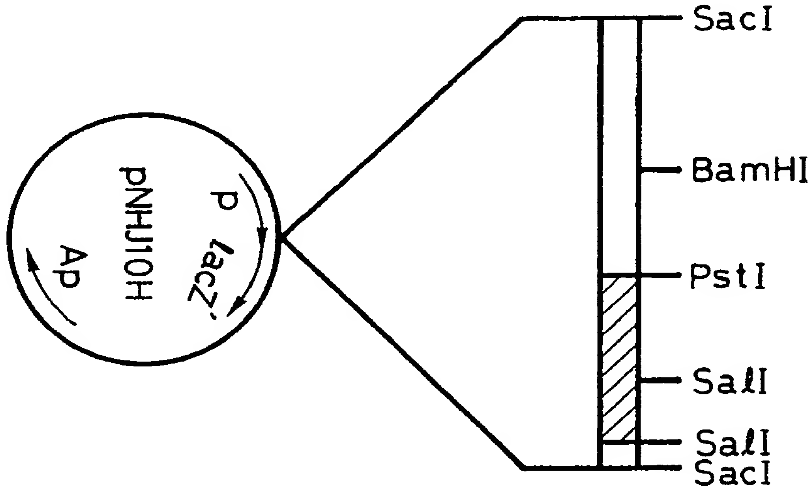
6. A transformant transformed with the recombinant DNA of claim 5.

7. A method of producing nitrile hydratase which comprises culturing the transformant as claimed in claim 6 and recovering nitrile hydratase from the culture.

8. A method of producing amides which comprises hydrating nitriles using nitrile hydratase obtained from the culture of the transformant of claim 6.

9. A method of producing amides which comprises culturing the transformant as claimed in claim 6, and hydrating nitriles to amides using the resultant culture, isolated bacterial cells, treated matter thereof, or a fixed material thereof.

FIG. 1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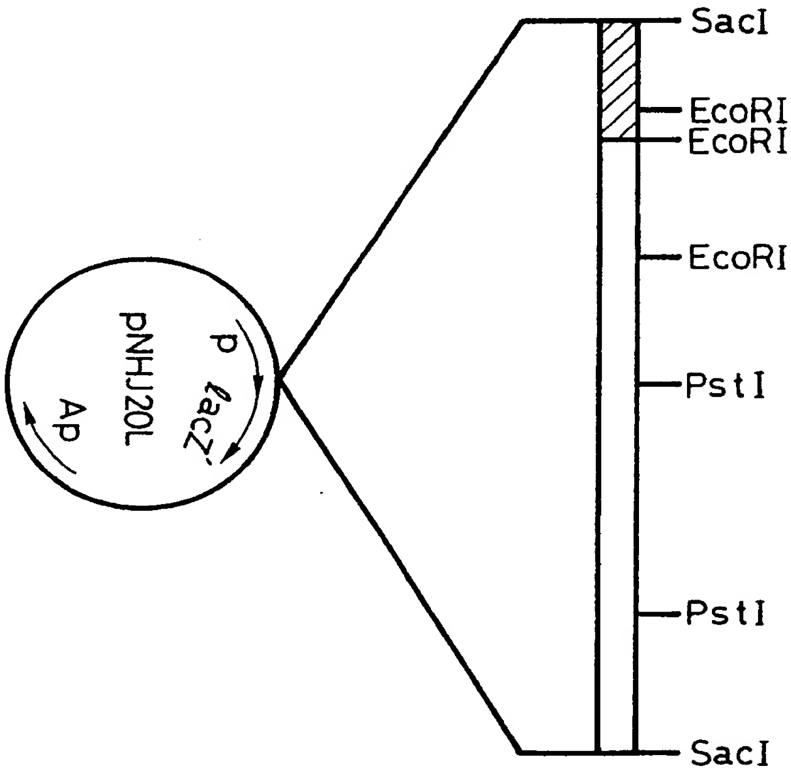
P lacZ'
Ap
SacI
BamHI
PstI
SalI
SalI
SacI


pNHJ20L
P lacZ'
Ap
SacI
EcoRI
EcoRI
EcoRI
PstI
PstI
SacI